Functional Food: Product Development and Health Benefits

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Abstract: Advances in food and nutrition sciences highlighted that diet can provide consumers with components able to both modulate body functions, improve health status and wellbeing and/or reduce the risk of some diseases. Therefore, much research has been undertaken in recent years on development and application of technologies to develop functional foods. Functional foods and dietary supplements can make health claims and/or structure/function claims. Health claims state that an ingredient may reduce the risk of a disease, whereas, structure/function claims declare that an ingredient in the product could benefit a body's structure (the skeletal system) or its function (the circulatory system). This paper includes information on the common products rich in nutraceutic components; explains the importance of probiotic and prebiotic foods and finally focuses on recent patents dealing with functional formulations for specific human diseases.

Keywords: Functional components, prebiotic, probiotic, functional food, nutrigenomics, health benefit.

1. INTRODUCTION

With increase use of dietary supplements and functional foods, questions have arisen about whether these products are safe to use and actually provide the health benefits they claim (United States General Accounting Office Washington, D.C. 20548 July 11, 2000). The International Food Information Service (USA) defines the functional foods as 'foods that provide benefits beyond basic nutrition' [1].

In the past, food technology has not been an area of frequent patenting relative to technologies that impact the nutraceutical and functional food markets, such as formulation science. Although patenting of food technologies is now on the rise, most of the filings are classified under those of medicines and usually cover processes such as extraction or purification rather than bare end product and therefore results in the FDA (US Food and Drug Administration) treating the products as drugs. To date, the FDA has only authorized health claims that reduce the risk of a disease. Interestingly, the FDA regulates nutraceuticals less stringently than foods in terms of what health claims can be made [2]. There is no FDA regulatory policy specific to functional foods; rather they are regulated under the same framework as conventional foods (Scientific substantiation of claims in the USA: focus on functional foods).

Both functional foods and dietary supplements can make health claims and/or so-called structure/function claims. Health claims state that an ingredient may reduce the risk of a disease whereas structure/function claims declare that an ingredient in the product will benefit a body's structure (such as the skeletal system) or function (such as the circulatory system) (United States General Accounting Office Washington, D.C. 20548 July 11, 2000). Japanese researchers introduced the term "functional food" more than twenty-five years ago, but the concept still lacks a uniform definition. The first book on this topic, [3] considers as functional ''any food that has a positive impact on an individual health, physical performance, or state of mind in addition to its nutritive values''. In other words, in addition to the bulk ingredients and calories that one consumes when eating food, a functional food has to deliver an additional beneficial effect on health or wellbeing. The Journal of Agricultural and Environmental Ethics [4] states that the two key aspects in the evaluation of functional foods are safety and efficacy. While safety can be covered under different legislative umbrellas such as novel foods (NFs), foods for particular nutritional purposes, supplements, additives and others, the issue of evaluation of their efficacy is only at a very early stage since the criteria to establish the validity of 'health claims' has not been clearly addressed at the European level. In addition, the European Journal of Clinical Nutrition [5] states that a functional food contains a component (whether or not a nutrient) that allows one to obtain benefits on body functions and physical wellbeing, psychological effect or reducing risk of disease. A group of European experts (Madrid, October 1998) adopted the following definition: "a food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects in a way which is relevant to either the state of well-being and health or the reduction of the risk of a disease". A functional food contains macronutrients or essential micronutrient for specific physiologic effects. It can also be stated that a food is functional if (i) it allows the elimination of known components that cause deleterious effects when consumed (e.g., an allergenic pro-

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tein); (ii) increases the concentration of a component naturally present in food to induce predicted beneficial effects (fortification with a micronutrient to reach a daily intake higher than the recommended daily intake but compatible with the dietary guidelines for reducing risk of disease); (iii) contains a component with known beneficial effects that is not normally present in most foods (e.g., non-vitamin antioxidant or prebiotic fructans); (iv) replaces a component (e.g., fats) whose intake is usually excessive, and thus represents a cause of deleterious effects, by a component for which beneficial effects have been shown (e.g., chicory); (v) increases the stability of a component known to reduce the potential disease-risk of the food [6].

Sustainability is a concept that emerged from the World Commission on Environment and Health (1987) and was developed further at the Earth Summit in Rio De Janeiro in 1992. Now it is even more broadly defined, extending beyond the status of resources to the health of communities, consumer empowerment and equity of access to material resources. Applications of this concept provide guidelines for decision-making and framework for assessing the impact of all initiatives. Given the food–environment–health links it embraces, it is particularly appropriate for assessing the impact of food-producing systems on individuals, communities and environment. Despite the developments, there are discrepancies between producers and consumers understanding and application of the concept of sustainability to systems of food supply [7]. So, assessing the sustainability of any type of food or food-related practice is problematic. Nonetheless, it is possible to list a range of criteria that can be applied to evaluate the sustainability of food systems, including functional foods.

In the first instance, food must provide nutrients and energy required to sustain wellbeing and reduce risks to physical health. The social dimensions of health require that individuals have the opportunities to express themselves and identify with others processes often involving the sharing of food. So, health is determined by the accessibility of food to consumers, and the opportunities for food choice, which translate to affordability, availability, variety and cultural relevance of food. They need to be able to decide the most cost effective way to protect their health when making food choices. This assumes that consumers are motivated and capable of accessing reliable information about the choices available to them, with implications for the ways complex science is translated into food labeling, advertising and other communications. In a sustainable system, consumers can influence decisions about food production as new scientific information about nutrition, their health needs and the environment emerges. Finally, producing functional foods must be cost effective in conventional terms, by comparing the resources consumed relative to the benefits they provide.

2. MAIN SOURCES OF FUNCTIONAL COMPONENTS

Cereals, legumes, fruit and vegetables are common products rich in nutraceutic components. Table **1** reports a summary of the other main functional components of foods and their benefits. At present gut health products in particular probiotics and prebiotics dominated the market of functional foods.

Probiotics are defined as live microbial food supplement that if consumed in adequate quantity can have beneficial effects improving the microbiological balance in the intestine. The microbial community in the large intestine is extremely complex, both in terms of the number of organisms, approximately 10^{13} cells/g contents in total, and in its diversity, with over 400 different species reported. This complex and highly adapted community also provides further benefits increasing resistance to pathogens. Because the native gut microflora are so well adapted to their environment, it is difficult for other organisms (including pathogens) to colonize the lumen. This creates colonization resistance or non-specific disease resistance in the gut, which increases resistance to pathogens. A variety of potentially harmful organisms are members of the normal gut microflora including bacteroides (some species are involved in putrefaction), clostridia and *Escherichia coli* (potential pathogens). These organisms are probably not considered harmful as part of the native microbial community, as their pathogenic effects are kept under control by the normal equilibrium of gut microbial metabolism. Disturbances in the normal intestinal microbial community structure can result in the proliferation of pathogens and cause gastrointestinal disorders [8]. Probiotic strains provide a number of benefits that have been reported or theorized. These benefits include: (1) re-establishment of balanced intestinal microflora and composition of intestinal flora, helping to increase the body's ability to resist the invasion of pathogens and maintain the host's wellbeing; (2) improving colonization resistance and/or prevention of diarrhea, urogenital diseases (*Candida vaginitis*), alleviation of constipation, protection against traveller's diarrhea, prevention of infantile diarrhea, reduction of antibody-induced diarrhea, control of inflammatory bowel diseases and irritable bowel syndrome; (3) systemic reduction of serum cholesterol confer health benefits to the cardiovascular system, including prevention and therapy of various ischemic heart syndromes; (4) reduction of fecal enzymes such as β-glucuronidase, β-glucosidase, nitroreductase and urease, potential mutagens which may induce tumors; (5) enhances lactose digestion and reduction of lactose intolerance; (6) enhancement of immune system response and prevent food allergy and atopic diseases; (7) improved calcium absorption; (8) synthesis of vitamins and pre-digestion of proteins. In addition, there have been reports of bacteriocins production by some probiotic bacteria, targeting pathogenic bacteria [8].

Lactic acid bacteria and bifidobacteria, the most studied and widely employed bacteria within the probiotic field, are normal component of the intestinal microbiota and have a long tradition of safe application within the food industry [9]. They are usually added to fermented dairy products that represent about 56% of functional foods [8].

The definition of prebiotics was updated in 2004. They are defined as ''selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" [10]. These components resist the hydrolysis of the digestive enzymes, because specific linkages between monomers are resistant against breakage by

Table 1. Main Functional Components of Foods and their Benefits

Class/Component	Potential Benefits
Inulin, Fructo-oligosaccharides (FOS), Polydextrose	**Prebiotics**: may improve gastrointestinal health; may improve calcium absorption
Yeast, Lactobacilli, Bifidobacteria, and other specific strains of beneficial bacteria	**Probiotics**: may improve gastrointestinal health and systemic immunity; benefits are strain-specific
Dietary (functional and total) Fiber	
Insoluble fiber	May contribute to maintenance of a healthy digestive tract; may reduce the risk of some types of cancer
Beta glucan	May reduce risk of coronary heart disease (CHD)
Soluble fiber	May reduce risk of CHD and some types of cancer
Whole grains	May reduce risk of CHD and some types of cancer; may contribute to maintenance of healthy blood glucose levels
Flavonoids	
Anthocyanins-Cyanidin, Delphinidin, Malvidin	Bolsters cellular antioxidant defenses; may contribute to maintenance of brain function
Flavanols-Catechins, Epicatechins, Epigallo-catechin, Procyanidins	May contribute to maintenance of heart health
Flavanones-Hesperetin, Naringenin	Neutralize free radicals, which may damage cells; bolster cellular antioxidant defenses
Flavonols-Quercetin, Kaempferol, Isorham-netin, Myricetin	Neutralize free radicals, which may damage cells; bolster cellular antioxidant defenses
Proantocyanidins	May contribute to maintenance of urinary tract health and heart health
Carotenoids	
β-carotene	Neutralizes free radicals, which may damage cells; bolsters cellular antioxidant defenses; can be made into vitamin A in the body
Lutein, Zeaxanthin	May contribute to maintenance of healthy vision
Lycopene	May contribute to maintenance of prostate health
Isothiocyanates	
Sulforaphane	May enhance detoxification of undesirable compounds; bolsters cellular antioxidant defenses
Phenolic Acids	
Caffeic acid, Ferulic acid	May bolster cellular antioxidant defenses; may contribute to maintenance of healthy vision and heart health
Sulfides/Thiols	
Diallyl sulfide, Allyl methyl trisulfide	May enhance detoxification of undesirable compounds; may contribute to maintenance of heart health and healthy immune function
Dithiolthiones	May enhance detoxification of undesirable compounds; may contribute to maintenance of healthy im-mune function
Plant Stanols/Sterols	
Free Stanols/Sterols	May reduce risk of CHD
Stanol/Sterol esters	May reduce risk of CHD
Phyto-estrogens	
Isoflavones-Daidzein, Genistein	May contribute to maintenance of bone health, healthy brain and immune function; for women, may contribute to maintenance of menopausal health
Lignans	May contribute to maintenance of heart health and healthy immune function

(Table 1) contd….

Class/Component	Potential Benefits
Fatty Acids	
Monounsaturated fatty acids (MUFAs)	May reduce risk of CHD
Polyunsaturated fatty acids (PUFAs) -Omega-3 fatty acids-ALA	May contribute to maintenance of heart health; may contribute to maintenance of mental and visual function
PUFAs-Omega-3 fatty acids—DHA/EPA	May reduce risk of CHD; may contribute to maintenance of mental and visual function
Conjugated linoleic acid (CLA)	May contribute to maintenance of desirable body composition and healthy immune function

mammalian enzymes and are not absorbed in the upper part of the gastro-intestinal tract, indeed pass into the large intestine where are located most of the indigenous intestinal microbiota. The presence of microbial enzymes able to act on these bonds allows these carbohydrates to be selectively degraded. Further selectivity is conferred by the fact that not all colonic microorganisms have the appropriate enzymes to degrade all linkages. The effect of a prebiotic is essentially indirect because the benefit to the host is mediated through selective stimulation of the growth and/or activity of one or a limited number of colonic bacteria, thus improving host health. It is not the prebiotic by itself but rather the changes induced in microflora composition that is responsible for its effects [11]. Bifidobacteria and/or lactobacilli are good target organisms [8].

A wide variety of dietary carbohydrates, especially fructo-oligosaccharide (FOS), galacto-oligosaccharides (GOS), inulin, isomalto-oligosaccharides (IMO), polydextrose, lactulose and resistant starch are considered as the main prebiotics components. These types of carbohydrates are not hydrolyzed by human digestive enzymes but are readily fermentable by specific colonic bacteria, such as bifidobacteria and lactobacilli species, with the concomitant production of short-chain fatty acids. These acids, especially butyrate, acetate, and propionate, provide metabolic energy for the host and acidification of the bowel [12]. FOS are chain-like carbohydrates consisting of a chain formed of fructosyl units with a glucosyl at the end, or fructosyl chain alone. By the chain length, i.e. the number of fructosyl units, FOS are divided into oligo-fructoses having a shorter chain, of which 1-kestose and nystose can be given as examples, an inulin having a longer chain (a common designation for FOS, which may contain up to 60 fructosyl units). Many cultivated vegetables, such as sugar-beet, chicory, Jerusalem artichoke, asparagus, onion, garlic, banana and tomato, contain FOS in abundance, however, FOS can also be produced synthetically. FOS are known to have a prebiotic effect, which means that they enhance selectively the growth of useful *Bifidobacteria* and *Lactobacilli* which produce acid in the intestines. FOS also reduce the pH in the intestines, thus inhibiting the growth of pathogen bacteria and maintaining optimal bacterial balance for human health. Other known effects of FOS on humans is an increase in excrements and a decrease in blood fat values and cholesterol level. Since digestive enzymes such as α-amylase, sucrase and maltase are not capable of decomposing FOS to any notable extent, and only the specific types of bacteria mentioned above utilize them selectively, FOS have been used as low-calorie sweetening agents in food. However, owing to the prebiotic and other useful effects mentioned above, FOS have a potential of being used more extensively especially as a food supplement to enhance intestinal activity. Inulin and oligofructose, non-digestible fermentable fructans, are amongst the most studied and well established prebiotics. These compounds have shown to increase calcium absorption, thus improve both bone mineral content and bone mineral density and reduce the risk of osteoporosis. Furthermore, delayed gastric emptying, modulation of gastro-intestinal transit times, improved glucose tolerance, reduced fat and cholesterol absorption via binding of bile acids, reduce the levels of cholesterol and serum lipids, reduce the risk of cardiovascular disease associated with dyslipidemia, especially hypertriglyceridemia and inulin resistance. Increased volume and water carrying capacity of intestinal contents, reduce the constipation relief resulting from fecal bulking and possible effects on intestinal motility and suppression of diarrhea, especially when associated with intestinal infections [6]. The oligosaccharides play an important role in the obesity control through increased satiety and reduced hunger.

Bifidobacteria have a number of health promoting properties, such as improved intestinal environment and help to restore the normal flora after antibiotic therapy, inhibition of pathogenic bacteria, immunomodulation, synthesis of B vitamins, improved calcium absorption, lowered blood ammonia and cholesterol concentrations and inhibition of tumor formation [8]. Lactobacilli may aid digestion of lactose in lactose-intolerant individuals, reduce constipation and infantile diarrhea, help to resist infections such as salmonellae and help to relieve irritable bowel syndrome [11]. Prebiotics can be used in a wide range of food products: dairy foods, baked goods, cereals based foods and meat products.

Symbiotics have also been defined as mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in its gastrointestinal tract. One of the main benefits of symbiotics is the increased persistence of probiotics in the gastrointestinal tract [13].

2.1. Cereal

Cereals, in particular oat and barley, are functional food, being sources of non-digestible carbohydrates as lactulose, FOS, trans-GOS, that have been shown to be effective in

stimulating growth of bifidobacteria and lactobacilli in human large intestine.

Cereals contain water-soluble fibers, such as β-glucan and arabinoxylan, that delay gastric emptying, reduce glucose and sterol absorption by the intestine, decrease total and low density lipoprotein, serum cholesterol, and consequently reduce the risk of coronary heart disease, postprandial blood glucose, and insulin contents [12]. The potential cholesterol lowering of cereal fibers is considered to result from effects manifest in the upper gastrointestinal tract. These in turn may be related to the ability of cereal fibers to form a gel-like network and alter gastrointestinal viscosity [14].

Some functional cereal components were also applied in dairy, pasta and bakery industries. Recent research has focused on the use of β-glucan in the manufacture of low-fat ice creams and yogurts. Incorporation of β-glucan with their soluble dietary fiber into low-fat dairy products can make their mouth-feel, scoop-ability and sensory properties resemble those of full-fat products [9].

2.2. Legumes

Legumes and soya-derived foods contain isoflavones, often referred as phyto-estrogens. Genestein and daidzein are the main isoflavones. Isoflavones are heterocyclic phenols structurally similar to the estrogenic steroids. Because of they are weak estrogens, isoflavones may act as anti-estrogens by competing with the naturally occurring endogenous estrogens. This may explain why populations that consume significant amounts of soya have reduced risk of estrogen-dependent diseases such as breast and prostate cancer. The consumption of foods rich in isoflavones may be associated with decrease risk of diseases. There is some evidence to suggest that isoflavones may be protective against hormone dependent cancers and against age-related diseases such as cognitive-decline, cardiovascular disease and osteoporosis. Isoflavones are extensively metabolised in the gut (including action of the gut microflora) and liver. Although cholesterol lowering is one of the best documented cardio-protective effects of soya, vascular protection may also contribute to improve cardiovascular health and is likely to be mediated by isoflavones via a number of mechanisms including effects on arterial function, cellular effects and estrogen-receptor mediated effects.

The U.S. FDA has contributed to a considerable growth in the sales of soya foods in the US since the year 2000. The FDA approved the claim that soya protein included in a diet low in saturates and cholesterol may reduce the risk of coronary heart disease by lowering blood cholesterol levels. The claim identifies 25g/day as the amount of soya protein required to produce health benefits. Currently, isoflavones are being used as bioactive ingredients in a wide range of functional foods from soya yogurts and puddings to tomato juice, containing soya germ. Indeed, a soya yogurt was developed using lactic acid bacteria and germinated soya bean extract that contains significantly higher levels of isoflavones, gamma-aminobutyric acid (GABA, the major inhibitory neurotransmitter in the central nervous system) and free amino acids than conventional soya yogurts, and consequently may have enhanced health benefits [15].

2.3. Fruit and Vegetables

The effects rendered by nutraceutical and functional foods are due to a cocktail of phytochemicals and bioactives present in fruit and vegetables. They are the principal source of such important nutrients as vitamins, minerals, folate, fiber, carotenoids, polyphenols, phytosterols. These compounds are essential bioactive compounds with antioxidant protection on health. Free radicals have also been implicated in the development of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, and in diabetes, rheumatoid arthritis, and chronic obstructive pulmonary and other diseases. These diseases may, therefore, be beneficially influenced by antioxidant consumption. The primary role of antioxidants in reducing the risk of cardiovascular disease is through inhibition of peroxidation in low density lipoprotein, although they may also influence other cardiovascular disease processes [16].

Several epidemiological studies have shown that fruits and vegetables are protective against a variety of human cancers. Tomatoes have received much attention in recent years because of interest in lycopene, the primary carotenoid in this fruit, and its potential antioxidant role in cancer risk reduction. Garlic (*Allium sativum*) contains an odorless amino acid, which is converted enzymatically by allinase into allicin when the garlic cloves are crushed. Allicin then decomposes spontaneously to form numerous sulfur-containing compounds (diallyl sulphide, diallyl disulfide), have been shown to inhibit tumor-genesis in several experimental models. Epidemiological studies also suggested that allium vegetables, including onions, may confer a protective effect on cancers of the gastrointestinal tract.

The tea, particularly green tea, is rich in polyphenols. Catechins are the predominant and most significant of all tea polyphenols. Tea epigalocatechin gallate, an important tea constituent, has strong antioxidant, immuno-stimulatory, anti-inflammatory and antimicrobial activities. Human intake of green tea decreases total cholesterol, increases HDL fraction and decreases lipoprotein oxidation [17]. Epidemiological evidence has also associated the frequent consumption of cruciferous vegetables with decreased cancer risk. The anti-carcinogenic properties of cruciferous vegetables have been attributed to their relatively high content of glucosinolates, a group of glycosides recordable in all cruciferous vegetables. Myrosinase, an enzyme found in plant cells, converts these compounds to a variety of hydrolysis products, including isothiocyanates, sulforaphane and indoles.

Several epidemiological studies have shown that citrus fruit are protective against a variety of human cancers. Although oranges, lemons, limes and grapefruits are a principal source of such important nutrients as vitamin C, folate and fibre, Elegbede *et al.* [18] have suggested that another component is responsible for the anticancer activity. Citrus fruit are particularly high in a class of phytochemicals known as the limonoids. Evidence has been accumulating in support cancer-preventative effect of limonene [19].

Another important products category within the functional food segment is non-alcoholic beverages fortified with vitamins A, C and E or other ingredients, eye health drinks (with lutein) or bone health drinks (with calcium and inulin) [9].

3. MAIN TECHNIQUES TO PRODUCE FUNCTIONAL FOOD

In the development of functional foods, it is essential to adopt an integrated approach which involves several interrelated elements functioning in harmony before the product can be successfully launched on the market. The process should start with considerations and decision-making about the product concept and positioning strategy, in particular with regard to regulatory positioning, labeling, etc. The next step is to identify chemically the bioactive components or ingredients which are considered to provide the desired beneficial effect on health. For example, modern liquid chromatography mass spectrometry analysis (LC/MS) can be applied for structural identification of target molecules. In the next step (product formulation), feasible technologies with appropriate analytical methods have to be applied or developed, if necessary, with the purpose of designing a product with the desired health-enhancing properties [20-22].

Many technological challenges and possible solutions for functional foods are proposed for example to eliminate harmful component(s) or to increase the concentration of beneficial indigenous components. To this aim, it is possible to use enzymatic processes, membrane separation or supercritical fluid extraction [23]. The last two techniques are also used for replacement of harmful components by beneficial components; whereas to increase bioavailability of beneficial components, microencapsulation technique may be adopted [24].

Microencapsulation is a technology based on packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rate under the influences of specific conditions (Table **2**). In a broad sense, microencapsulation can be used for many applications in food industry, including stabilizing the core material, controlling the oxidative reaction, providing sustained or controlled release, masking flavors, colors or odors, extending the shelf life and protecting components against loss [25].

Nanotechnology represents the most recent field with great potential in improving the efficiency of release of nutraceuticals and bioactive compounds in functional foods to improve human health [26]. Some of potential delivery systems based on nanotechnology are association colloids, nano-emulsions, nanostructured multiple emulsions, and also, nanocomposites, nanofibers and so on [27].

To improve retention of beneficial components in raw materials and foods encapsulation processes, pulsed electric field and sphere packaging have been also developed [23].

Such processes include the following aspects [28]:

- Elimination of a component known or identified as causing deleterious health effects, e.g. allergenic proteins;
- Increase of concentration of natural bioactive component in the food, up to the desired effective level;
- Addition of a component which is not normally present in most foods, but for which beneficial effects have been demonstrated;
- Replacement of a component, usually a macronutrient, the intake of which is considered harmful to health in high intake amounts, by a component for which the beneficial effect has been demonstrated;
- Improvement of the bioavailability or modification of food components for which beneficial effects have been demonstrated;
- Monitoring amount and efficacy of beneficial bioactive components

The accurate identification of bioactive compounds is essential to identify relationships between different food components and health benefit. In addition, precise quantification is critical to determine dietary intake levels and safety guidelines for potentially bioactive compounds necessary to achieve desired beneficial properties. Rapid analytical methods are needed to monitor the biomarkers influencing the disease state to effectively demonstrate benefits of this added value for consumers. The four common steps for any analytical method are sampling, sample preservation, sample preparation, and analysis (separation and detection) [29]. Over 90% of developments made during the past few decades has focused on the final step of analysis. Remarkable advances in instrumentation, spectroscopy, and chromatography have resulted in rapid advancement of methods for high-throughput separation and detection of complex multicomponent mixtures containing trace quantities of the analytes of interest. The sampling is the collection of a representative sample from the entire matrix that needs to be analyzed. The sample is obtained in such a way that it truly represents the entire sample. Sample preservation is an impor-

Table 2. Techniques and Types of Materials for Encapsulating Probiotic Microorganisms

Microencapsulation	Types of Materials for Coating
Spray-drying	Water-soluble
Spray-congealing	Waxes, fatty acids, water-soluble and water-insoluble polymers, monomers
Fluidized-bed coating/ air-suspension	Water-insoluble and water-soluble polymers, lipids, waxes
Extrusion	Water-soluble and water insoluble dehydrating liquid polymers
Coacervation/phase separation technique	Water-soluble polymers
Electrostatic method	Oppositely charged polymers/ Compounds

tant step as there is often some delay between sample collection and analysis. The proper sample preservation ensures that sample retains its physical and chemical characteristics from the time it is collected to the time it is analyzed [29]. Therefore, it is essential to inactivate all enzymatic, metabolic, and chemical reactions during preservation to maintain accurate sample identity, and demonstrate the effectiveness of such procedures in any report. Thus, researchers need to study in detail the influence of storage temperature on the analyte of interest and preserve samples under appropriate conditions. Sample preparation consists of multiple steps such as sample drying, homogenization, sieving, extraction, pre-concentration, derivatization, and hydrolysis. The motive behind sample preparation can be multi-fold: to increase the efficiency of an assay procedure, to eliminate or reduce potential interferences, to enhance the sensitivity of the analytical procedure by increasing the concentration of the analyte in the assay mixture, and sometimes to convert the analyte of interest to a more suitable form that can be easily separated, detected, and/or quantified [30]. Once the sample preparation is completed, the analysis is carried out by selected instruments. A variety of instruments are used for different types of analysis, depending on the information to be acquired. There is seldom a unique way to design a measurement process. Even an explicitly defined analysis can be approached in more than one ways. The objective of an analytical measurement can be qualitative or/and quantitative [29].

4. RECENT TEST-PATENTS

4.1. Diabetes Preventing

The document EP1920774A1 describes the composition for treating cancer, viral infection, allergic disease, autoimmune disease, inflammatory diseases or diabetes [31]. This invention relates compositions based on fucoidan or a fucoidan hydrolysate and an immuno-stimulating material that can be used in foods, beverages, pharmaceutical, health foods, functional foods and cosmetics. It also helps to reduce the time taken to select lactic acid bacteria that have a strong immuno-stimulating activity. To this aim, fucoidan or a product obtained by acid hydrolyzing fucoidan is used in combination with an immuno-stimulating material. This combination synergistically potentiates the immuno-modulatory or immuno-stimulating activities of the ingredients. The immuno-modulatory activity as used herein does not mean indirect regulation of immuno-function through improvement of enterobacteria, as achieved by xylo-oligosaccharides, but means direct activation of immuno-competent cells as exhibited by several kinds of fucoidan.

Another objective of the present invention is to provide a composition, an effective amount of which can be added in a precise manner to foods/beverages or pharmaceuticals. Fucoidan or a hydrolysate and the immuno-stimulating material that are combined in accordance with the present invention offer the advantage of exhibiting an enhanced immuno-modulatory or immuno-stimulating action, compared with their individual application. This makes it possible to develop foods, beverages and pharmaceuticals that are more effective than products that incorporate the conventional immuno-stimulating materials. Such high efficacy contributes to a reduction of the amount of immuno-stimulating material to be added. These advantages bear particular importance when lactic acid bacteria are used as immuno-stimulating material. For instance, lactic acid bacteria of low water solubility may be prevented from precipitating if the amount of their inclusion is reduced. In addition, the step of selecting lactic acid bacteria that have both immuno-stimulating activity and desired fermentation characteristics can be skipped. As Fucoidan or its hydrolysate is isolated from a natural foodstuff, its effect is mild but with a very high degree of safety. Fucoidan derived oligosaccharides have low molecular weights, so they are easy to handle and can be manufactured in a simple manner; in addition, because of their high safety, such oligosaccharides may be combined with immuno-stimulating materials to prepare functional formulations.

The document US7618951B2 has as an object of invention the development of food containing prebiotics, preferably gluco-oligosaccharides, or pharmaceutical compositions for preventing onset of type II diabetes mellitus in predisposed patients [32]. The invention relates to the use of prebiotics for the preparation of food or pharmaceutical compositions which are intended for the treatment and/or prevention of hyper-glycemic syndromes and, in particular, for the treatment of Type II diabetes and/or the prevention of the onset of Type II diabetes in patients with a predisposition to developing diabetes. The invention also relates to the food and pharmaceutical compositions containing the prebiotics. Prebiotics are non-digestible dietary compounds broken down by the microorganisms of the intestinal flora and whose breakdown is responsible for certain health benefits. These beneficial health effects are due to a selective stimulation of the growth and/or the biological activity of certain microorganisms of the intestinal flora, in particular the bifidobacteria and the lactic acid bacteria of the colic flora. The effects of prebiotics are principally due to stimulation of the growth of bifidobacteria (bifidogenic effect). The stimulation of this growth allows a reduction in the pH of the colon, an increase in the production of short chain fatty acids, in particular butyrate and propionate, a prevention of the installation of pathogenic microorganisms (barrier effect), an increase in the metabolization of potentially carcinogenic compounds and the production of vitamin B. The use of prebiotics also allows stimulation of the immuno-system due to the production of lipoteichoic acid by the bacteria, the interaction of these bacteria with the Peyer' s patches and the stimulation of peripheral lymphocyte circulation. Prebiotics also encourage the digestive absorption of minerals, in particular calcium and magnesium, which allow their potential application in the context of the treatment of osteoporosis. In the conditions under study the prebiotics that are clearly defined are sugars classed among dietary fibers: non-digestible oligosaccharides (also called oligosides). Oligosaccharides are monosaccharide polymers with a low degree of polymerization. The number of osidic units is typically from 2 to 12 units with an average of approximately 3-5 units. The monosaccharides involved in the formation of oligosaccharides are varied, in particular hexoligosides such as glucose, galactose, and fructose, but also pentoligosides such as xylose are found. Oligosaccharides can be derived from the breakdown of natural polymers, such as starch or

insulin, by direct extractions from natural substances, such as soybean, or by chemical or enzymatic syntheses. Gluco-oligosaccharides (GOS) constitute an important class of oligosaccharides. The present invention also demonstrates that the glycemia of mice suffering from type II diabetes could be reduced through a treatment based on prebiotics. Thus, the principal aim of the invention is to provide functional food, nutraceuticals or medicaments, intended for the treatment and/or the prevention of hyperglycemic syndromes and in particular for the treatment of type II diabetes and/or the prevention of the appearance of type II diabetes in at-risk subjects. An aspect of the invention is also food composition, nutritional additives, functional food, or nutraceuticals, comprising one or more prebiotics, intended for the nourishment of subjects suffering from hyperglycemic syndrome and/or at risk of developing this syndrome. The invention also relates to any pharmaceutical composition that is characterized by one or more prebiotics in combination with a pharmaceutically acceptable vehicle. The tests carried out by the inventors showed that the excess weight of the control and treated animals were similar and the fatty deposits were equivalent. Moreover the glicosuria and the fasting glycemia were normal, thus indicating no signs of obesity-related diabetes. The glucose tolerance test by contrast indicated that the control mice presented a glucose resistance while this phenomenon was significantly corrected in the treated mice. Glucose intolerance is considered to be one of the first symptoms of the appearance of type II diabetes. It therefore appeared that the treatment with gluco-oligosaccharides was sufficient to prevent the appearance of this diabetes in a murine obesity model.

The document US7422764B2 describes an edible liquid composition for treating or preventing disease or disorder, e.g. overweight and diabetes type II in mammal [33]. It comprises pectin and alginate, calcium, and indigestible oligosaccharide. The edible liquid composition based on pectin, calcium and indigestible oligosaccharide needs proper characteristics in terms of pH, viscosity, temperature and shear rate. Another aspect of the invention relates a method for the treatment or prevention of overweight or obesity in mammals. Obesity is a major health problem; approximately ninety-seven million people are considered clinically overweight in the United States. Various chemical approaches have been proposed for controlling obesity. Anorectic agents such as dextroamphetamine, the combination of the non-amphetamine drugs phentermine and fenfluramine (Phen-Fen), and dexfenliuramine (Redux) alone, are associated with serious side effects. Indigestible materials such as olestra, mineral oil or neopentvl esters have been proposed as substitutes for dietary fat. Garcinia acid and derivates thereof have been described as treating obesity by interfering with fatty acid synthesis. Surgical techniques such as temporary ilcal bypass surgery are employed in extreme cases. However, methods for treating obesity, such as those described above have serious shortcomings. Controlling the diet remains the most prevalent technique for controlling obesity. Hence, new compositions suitable for the treatment of obesity are highly desired. The accumulation or maintenance of body fat bears a direct relationship to caloric intake. Therefore, one of the most common methods for weight control to combat obesity is the use of relatively low-fat, high fiber food. Especially high viscosity fibers may advantageously be employed in anti-obesity diets. The high viscosity fibers may induce a sensation of satiety when ingested in a sufficient amount. The present invention relates to a composition that is liquid at around neutral pH and forms a viscous matrix at low pH. The composition comprised pectin, calcium and oligosaccharides and is particularly suitable for use in a method of treating or preventing overweight or obesity. Thus, the present invention also encompasses the method of treating overweight or obesity in mammals, by the intake of an effective amount of the aforementioned composition. Calcium is essential in the diet. Calcium deficiency may result in the following symptoms: muscle cramps, brittle nails, eczema, aching joints, increased cholesterol levels, rheumatoid arthritis, tooth decay and numbness in the arms and/or legs. Additionally, it is of importance in compositions, which are used to prevent or treat overweight and obesity. It has been described that calcium contributes to the prevention of obesity when absorbed by the body. Consequently, there are severe limitations to the use of low methoxylated pectins in compositions designed to reduce body weight and/ or to prevent body weight increase due to the impact of pectin and/or alginate on the bioavailability of calcium. The ingestion of nutritional compositions, including those that are designed to prevent or treat excessive body weight, should not become an undue burden. Humans participating in a weight control program including a specific diet often prematurely discontinue the program due to the adverse taste, insufficient palatability or difficulty of consuming the dietary components. The inventors experienced that compositions in liquid form, are preferred by subjects participating in a weight control program. It is therefore a prerequisite that a composition designed to reduce body weight or control the caloric intake is provided in a liquid form, with limited viscosity. However, this provides an additional challenge, since calcium and low methoxylated pectins form a viscous mass when present in an aqueous environment. It was found by the inventors that the calcium absorption inhibitory effect of low methoxylated pectins and/or alginate present in edible liquid composition can be decreased effectively through the co-administration of calcium and indigestible oligosaccharides with a degree of polymerisation between 2 and 60. The present liquid edible composition uses a calcium salt that provides only limited amounts of free calcium ions at around neutral pH and an increased amount of free calcium ions at acidic pH. The present composition thus provides a composition which is easy to consume, which induces feelings of satiety after ingestion and which ensures sufficient calcium bioavailability. A consumer may easily prepare the present edible liquid composition from e.g. a powder that contains pectin and/or alginate, calcium and oligosaccharide by simply adding a predetermined amount of water and/or milk. The suitable solvent preferably has a pH above 6 and may properly be selected from the group consisting of water, milk and mixtures thereof. Preferably, the mixture for making the present edible liquid composition is in the powder form. The invention also provides a process for preparing the liquid composition, comprising admixing the aforementioned mixture with a solvent that is preferably selected from the group consisting of water, milk and mixtures thereof. The term "reconstitutable form" as used herein refers to a preparation that needs addition of a suitable liquid (preferably water) to obtain the

present edible liquid composition. Preferably the "reconstitutable preparation" is a powder. The present composition may also be in a ready to drink form, and can be consumed without further preparation, i.e. does not require the addition of water before ingestion. The specific combination of the ingredients of the present composition also ensures a good shelf life.

The document US20100285156A1 has as an object of invention the development of functional food for improving or alleviating metabolic diseases e.g. diabetes, hyperlipidemia, arteriosclerosis, cardiovascular diseases and/or fatty liver, comprises extract of *Lysimachiae foenumgraeci* herb [34]. Disclosed are raw material, functional foods, and herb medicines for preventing and treating metabolic diseases, which comprises a *Lysimachiae Foenum-Graeci* herb extract as an effective ingredient. The extract is able to reduce blood glucose levels, triglycerides and cholesterol levels, AST (Aspartate Aminotransferase – Liver Function Tissue Damage) and ALT (Alanine aminotransferase – Liver Function Cell Damage) levels, and fat in the liver, and thus can be effectively used as a hepatic protector and a remedy for preventing and treating various metabolic diseases including diabetes, high blood pressure, fatty liver, cardiovascular diseases, and hyperlipidemia.

The patent US20100144668A1 mentions semi-fluid foodstuff comprising guar gum and β-glucan fibers per portion of foodstuff, useful to prevent diabetes, obesity, and cardiovascular disease, to reduce postprandial insulinemia and as functional food [35]. The invention also relates to novel food products including dietary fibers (guar gum and β-glucan fibers) intended to decrease the insulinaemia response. The ingestion of a typical meal, supplying proteins, lipids and carbohydrates, is quickly followed by an increase in glycaemia due to the absorption of carbohydrates. Glucose and fatty acids (FAs) are the body's two principal sources of energy and their uses are interconnected. Their respective degree of use is determined by insulin. The intensity of the oxidation of FAs is determined by their concentration in the blood and that of glucose would follow the same law in the absence of insulin. The balance between carbohydrate oxidation and lipid oxidation is thus achieved on the basis of simple competition for substrates, taking into account that the use of glucose is given priority by insulin, which induces its secretion [36]. The situation immediately postprandial (the first two hours after a meal) is a combination of hyperglycaemia and the hyper-insulinaemia it causes. A consequence of this hormonal change is a stimulation of the use of glucose by insulin-sensitive tissues, principally by an increase in glucose transport in cells. In addition, any rise in the concentration of insulin in the blood corresponds to a drop in the concentration of circulating FAs. Indeed, insulin inhibits the mobilization of stored lipids and favors their storage in fat tissues [37, 38]. Current consumption habits supply a higher quantity of energy than the quantity of energy expended daily. The body, due to higher insulin secretion, stores this surplus energy. Therefore, to remediate this situation, the consumption of energy beyond daily energy requirements must be limited. One needs to decrease the fat content of food, as well as to reduce insulin secretion by modifying the supply of carbohydrates in such a way that they cause less insulin secretion. The inventors aimed to identify ingredients to decrease the insulinaemia response of a meal in subjects, in particular healthy subjects of normal weight or overweight subjects (20<BMI<30) while maintaining a relatively low glycaemic response. Numerous studies on the impact of soluble dietary fibers were carried out. Studies of note include those of Spiller *et al*. [39] that described the hypolipidaemia effect of fibers of guar gum or of 3-glucan and the effect of guar gum and oat fibers source on plasma lipoproteins and cholesterol in hyper-cholesterolemic adults [39]*;* the study of Begin *et al.* [40] which described the effect of soluble dietary fibers (guar gum, carboxymethylcellulose, mustard mucilage, or oats) on glycaemia and insulinaemia; the study of Vachon *et al.* [41], which describes that soluble dietary fibers have a positive effect on postprandial insulinaemia but little effect on glycaemia.

The document WO2010098609A2 describes the pharmaceutical composition useful for prevention and treatment of diabetes mellitus comprises shikonin compound as an active ingredient, carrier, and additive or excipient [42]. The present invention relates to a composition comprising shikonin compounds, especially, isobutyryl shikonin, β-β-dimethylacryl shikonin, isovaleryl shikonin, and α-methyl-n-butyryl shikonin as active ingredients for prevention and treatment of diabetes mellitus. The composition of the present invention showed stimulating effect on the release of insulin in the beta cell of the pancreas due to inhibitory effect on K-ATP ion channel of beta cell in pancreas together with promoting effect on the increase of calcium concentration. Therefore, it can be used as the therapeutics, health functional food or food additive for treating and preventing diabetes mellitus.

The document US7772212B2 describes the useful composition for decreasing population of pathogenic bacteria e.g. *Escherichia coli* in bird's intestine, comprising commercial avian food and at least one maltosyl-isomaltooligosaccharide with specific linkages at specific concentration of IMO to inhibit avian pathogenic intestinal bacteria [43]. IMO produced by *Leuconostoc mesenteroides* ATCC 13146 fermentation with a sucrose:maltose ratio of 2:1 have been discovered to be effective prebiotics in mixed cultures of microbial populations. Surprisingly, in mixed microbial cultures this IMO composition proved to be effective as FOS. Thus, it can be used as effective prebiotic formulation for both birds and mammals. Moreover, the IMO were discovered to be effective non-competitive inhibitors of α-glucosidase, thus becoming useful in a therapeutic application for several diseases, including obesity, diabetes mellitus, pre-diabetes, gastritis, gastric ulcer, duodenal ulcer, caries, cancer, viral disease such as hepatitis B and C, HIV, and AIDS. A diet with 5-20% IMO was also shown to reduce the abdominal fat tissue in mammals. This invention pertains the use of maltosyl-IMO as a dietary supplement specifically for birds and mammals to promote the growth of beneficial intestinal microbes, inhibit the growth of pathogenic intestinal microbes, and for therapeutic intervention during diseases such as diabetes. As previously stated, prebiotics are non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial microbial strains (probiotics) residing in the host intestine [44]. It is believed the ability of these probiotics to catabolize oligosaccharides (two to ten monosaccharide units linked with glycosidic bonds),

to bestow beneficial health effects. Certain oligosaccharides are used as prebiotics. They are resistant to metabolism and adsorption in the small intestine and positively influence the composition of microflora in the large intestine. Oligosaccharides used as prebiotics are currently produced either by extraction from plant sources, acid or enzymatic hydrolysis of polysaccharides or enzymatic synthesis by transglycosylation reactions. They are composed of a D-glucopyranose unit at the non-reducing end (G) linked via an α-1,2 linkage to two or more 13-2,1-linked fructosyl units (F). This group includes 1-kestose (GF2), nystose (GF3), and 1 F-fructofuranosyl nystose (GF4). Many of the oligosaccharides marketed commercially are FOS, e.g., Raftilose and Nutraflora in the United States. GOS, which are α-galactosyl derivatives of sucrose, are present in many legume seeds. Mono-, di-, and tri-α-galactosylsucrose, known respectively as raffinose, stachyose, and verbascose, are produced by extraction from plants, particularly soybeans. These oligosaccharides are known to be, in part, responsible for the flatulence and diarrhea that follows consumption of beans, because of the absence of α-galactosidase in the gastrointestinal tracts of humans and animals. Gluco-oligosaccharide (GOS) is a generic term for poly-glucose oligomers. GOS may contain a number of different linkages and are generally obtained from starch hydrolysates (maltose and maltodextrins) through the action of the α-transglucosidase (EC 2.4.1.24) from *Aspergillus* spp. Gluco-oligosaccharides can also be produced by restricting polymer size during the fermentation process. A subcategory of GOS is the IMO, which contain α-1.6 bonds in their main chain. Ingested oligosaccharides (prebiotics) are capable of reaching the colon without being digested. It has been proposed that *Lactobacilli* and *Bifidobacteria*, which are considered beneficial species of the human intestinal tract, preferentially utilize FOS. Substituting FOS as a carbon source would preferentially increase the concentration of *Lactobacillus* and *Bifidobacteria* species with a concomitant rise in the intestinal production of lactic acid and short-chain fatty acids (SOFA). Both these products would have the net effect of lowering the pH in the large intestine. FOS, GOS, and soy-bean oligosaccharides were found not to be digested by enzymes secreted by small intestine, but to be fermented by certain microorganisms found in human and livestock intestines, especially by the *Bifidobacterirum* spp. Starch is one of the most readily available fermentable sources of energy for organisms and makes up 60-70% of the dietary carbohydrate consumption in humans. Humans secrete a pancreatic α-amylase that cleaves starch to a di-(maltose), tri-(maltotriose), and branched α-dextrins in the duodenal cavity. As there is no integral transport process in the intestinal enterocyte that can accommodate anything larger than free glucose, these oligosaccharides are further processed to glucose in the intestinal surface membrane by α-glucosyl saccharidases, including α-glucosidase. These enzymes form part of a large glycoprotein component of the intestinal surface brush border membrane. Once formed, glucose then may be co-transported into the enterocyte. Inhibitors of α-glucosidase are known to delay the digestion of starch, of starch-derived oligosaccharides, and sucrose (U.S. Pat. Nos. 5,840,705; and 4,013,510; and U.S. Patent Application No. 200410081711). At least two commercial oral α-glucosidase inhibitors, miglitol and acarbose, are currently prescribed for use in managing non-insulin-dependent diabetes mellitus by slowing the appearance of glucose in the blood after eating.

4.2. Anti-Obesity

The document WO2010064845A2 describes the composition useful for preventing and treating obesity, hypertension, and hyperlipidemia, based on *Myristica fragrans Houttuyn* extract [45]. The extract may be used for the prevention and treatment of obesity caused by the intake of high-fat diets, the increase in weight of periovarian fats and the accumulation of abdominal fats caused by the hypertrophy of adipocytes (fat cells).

The document US7790702 mentions protein/ phospholipid bonded substances that improve lipid and cholesterol metabolism for treatment of hypertension, obesity, fatty liver etc [46]. The present invention relates to a protein/ phospholipid or protein hydrolyzate/phospholipid complex containing 10 wt % or more of bound phospholipid, a lipid metabolism improving agent and a functional food comprising the complex.

The document WO2010104242A1 discloses new modified *Lactobacillus* FARM1, FARM2 and FARM3 strains, useful in pharmaceutical composition and functional food for preventing and treating obesity and metabolic diseases induced by obesity [47]. The present invention relates to prevention and treatment of obesity and metabolic diseases induced by obesity, particularly for the prevention and treatment of obesity by improving the characteristics of intestinal bacteria. In the present invention, it was ascertained that the characteristics of intestinal bacteria are transformed by the intake of a microbial preparation that reduces free fatty acid absorption in the gastrointestinal tract. A modified *Lactobacillus* strain usable for such purposes is provided. The present invention shows a weight loss effect equal to that of orlistat that is the most widely used anti-obesity therapeutic agent.

The document WO2010013978A3 describes the composition to prevent or treat hyperlipidemia, fatty liver, cardiovascular disease or obesity, comprises sialic acid compounds [48]. The invention relates to a composition for the prevention or treatment of hyperlipidemia, fatty liver, cardiovascular disease, or obesity, which includes a compound expressed by the following general formula I as its active ingredient:

General formula [I]: S-(MS)p-(MS)q where S represents sialic acid, and (MS)p and (MS)q monosaccharide residue, respectively.

The compound used as active ingredient causes weight loss, reduces organ fat, reduces total cholesterol concentration, reduces LDL cholesterol value, and reduces leptin value, thus exhibiting positive activity in prevention or treatment of hyperlipidemia, fatty liver, cardiovascular diseases, or obesity. In addition, because the composition of the present invention is free of cytotoxicity and skin adverse reactions, it may safely be applied in pharmaceutical and functional food compositions.

The document US20100151057A1 describes a pharmaceutical compound for use as raw material, functional food, cosmetic or crude drug for preventing and treating obesity,

based on extract of *Lysimachiae Foenum-graecum* herb [49]. An anti-obesity active extract obtained from a medicinal plant was disclosed. The extract can suppress the adipocyte differentiation and decreases body weight and body fat in an obese animal, thereby suppressing fat accumulation. Obesity is a disease caused by a metabolic error, in which excessive energy in a body is converted into fat and the fat is excessively accumulated in adipose tissue, thereby abnormally increasing body fat. Factors causing obesity are largely divided into genetic factors and environmental factors. The present invention provides a raw material, a functional food, cosmetic, a crude drug, etc. for an obesity preventing or therapeutic agent, in which the extract having a significant effect on suppression of adipocyte differentiation and a decrease in body weight and body fat in an obese animal model is included as an active ingredient. The invention also provides the method of extracting the extract from the *Lysimachiae Foenum-graecum* herb. The method included the following steps: (1) drying and grinding the herb; (2) carrying out solvent extraction by adding an organic solvent in an amount of 5 to 50 times by weight of the herb obtained in the first step; and (3) filtering extract solution of the organic solvent by using a filter paper, and carrying out vacuum concentration at temperature of 40°C or less. The composition according to the present invention includes the extract in an amount ranging from 0.1 to 50%, based on the total weight of the composition. The composition may additionally include a carrier, an excipient, or a diluent. A solid preparation for oral administration includes tablet, pill, powder, granule, capsule, etc., and such a solid preparation may be prepared by mixing the extract with at least one excipient (such as starch, calcium carbonate, sucrose, lactose, gelatin, etc). Also, in addition to the excipient, lubricants, such as magnesium stearate, and talc, may be used. A liquid preparation for oral administration includes suspension, liquid for internal use, emulsion, syrup, etc., and in addition to a frequently used main diluent, such as water and liquid paraffin, the preparation may include a variety of excipients (for example, a wetting agent, a sweetening agent, an aromatic agent, a preservative, etc). A formulation for parenteral administration includes a sterile aqueous solution, a non-aqueous solvent, a suspension, an emulsion, a freeze-dried preparation, and a suppository. The current patent also provides a health care food for preventing an anxiety disorder related to a cranial nerve system, which includes the *Lysimachiae Foenum-graecum* herb extract and an acceptable food supplement additive. It may be added to various foods and may be used in the form of pills, powders, granules, infusion, tablets, capsules, or drinks. The food supplement additive defined in the present specification includes food additives conventionally known as fragrance agents, flavoring agents, coloring agents, fillers, stabilizers, etc. Besides the extract as essential ingredient in a predetermined quantity, the health drink composition may include other ingredients without particular limitations, and may include various fragrance agents, natural starch, various nutrients, vitamins, minerals, flavor agents, coloring agents, extenders (cheese, chocolate, etc.), pectic acid and salt thereof, alginic acid and salt thereof, organic acid, protective colloid thickeners, pH adjuster, stabilizer, preservatives, glycerin, alcohol, and carbonating agents.

The document US7704979 also reports the method for treating, reducing or attenuating obesity [50]. The current invention provides methods of inducing the loss of adipose tissue by providing a diet high in calcium. In one aspect of the invention, the calcium is provided in the form of dairy products. In yet another aspect of the invention, calcium is provided in the form of a dietary supplement, such as calcium carbonate of vitamin supplements. Methods of suppressing $[Ca^{2+}]i$ levels in individuals are also provided. The invention also provides methods of stimulating lipolysis, inhibiting lipogenesis, and increasing the expression of white adipose tissue uncoupling protein 2. Moreover, it also provides methods of increasing the core temperature of an individual. The regulation of body weight, and particularly body fat in animals, is a complex process having enormous implications for the health and wellbeing of humans and other animals. Excess body weight and/or excess of body fat relative to lean body mass have been associated with a wide range of health problems including coronary artery diseases, stroke, and diabetes. It has been estimated that half of all American people are over-weight. Within the United States about 24% of men and 27% of women are defined as mildly to severely obese. Individuals 20% over ideal weight guidelines are considered obese. Obesity is classified as mild (20-40% overweight), moderate (41-100% overweight), and severe (>100%) overweight. Severe obesity is relatively rare, affecting less than 0.5% of all obese individuals and about 0.1% of the total population. Obesity is not just a problem for humans. Therefore, it is critical to maintain a healthy weight in order to minimize disease risk (U.S. Pat. No. 6,071,544). Obesity in humans is treated by a variety of means ranging from surgical procedures (gastric bypass) for the severely obese to diet therapy, behavior modification, and medication for the mildly to moderately obese. Management of moderate and mild obesity is typically performed by the individual and commercial organizations that provide behavior modification programs and, in some cases, prepackaged diets. The medical community recommends that diet treatments should be administered under medical supervision. The range of treatments for obesity reflects the complexity of the processes involved in weight regulation and the current lack of understanding of these processes. Recent reports have even implicated viruses as a possible causative factor in obesity (U.S. News and World Report, Aug. 7, 2000). There are also numerous reports of possible genetic bases for a predisposition to obesity. The present invention also provides methods for attenuating weight and adiposity in children and for inducing the loss of adipose tissue in humans or other animals by increasing the amounts of dietary calcium. Novel and advantageous methods of restoring normal body fat ratios in women post partum are also provided. This is put in practice by identifying an individual who has recently given birth to a child and increasing the amount of dietary calcium consumed by the individual. Methods of preventing or reducing the regain of weight lost after an initial period of dieting are also provided by the current invention. Methods of reducing, treating, or attenuating obesity in an individual comprising the administration of therapeutically effective amounts of 1,25-$(OH)_2$-D receptor antagonists are also suggested. Calcium provided to an individual in accordance with the subject invention may take the form of, for example, a dairy product (for example milk, no-fat dry milk,

yogurt, cheese, cottage cheese, ice cream or frozen yogurt), a nutrient supplement (such as calcium fortified vitamins and liquids supplemented with calcium), foodstuffs supplemented with calcium, or other foods high in calcium (for example, salmon, beans, tofu, spinach, turnip greens, kale, broccoli, waffles, pancakes, or pizza). Recommendations regarding increases in the amount of calcium consumed by the individual, as well as sources of dietary calcium, may be provided from a database that compares the amount of calcium consumed with that found to optimize or induce weight loss. The weight/height ratio may be calculated by obtaining the weight of an individual in kilograms and dividing this value by the height of the individual in meters. Alternatively, the weight/height ratio may also be calculated by multiplying the weight of the individual in pounds (lbs) by 703 and dividing this value by the square of the height of the individual (in inches). These ratios are typically referred to as BMI. Thus, BMI=kg/m^2 or BMI=(lbs.x703)/$(in)^2$. Information related to the benefits of maintaining a normal weight, or optionally a normal weight/height ratio, dietary plans containing high levels of calcium and printed matter disclosing the benefits of a high calcium diet may, optionally be provided in electronic or printed form and may be stored in a database. A further aspect of this invention is the method for promoting good health by providing a product with calcium accompanied by information regarding the benefits of the consumption of calcium with respect to the control of obesity. The information regarding the benefits of the consumption of calcium would typically include, for example, a written or verbal explanation that the consumption of calcium is associated with weight loss and/or the prevention of weight gain. This information can be presented in such a way so that a potential purchaser and/or user of a product which contains calcium would understand that the consumption of the product with calcium could cause weight loss or reduce weight gain, and that the weight loss or reduction in weight gain from the use of the product would be directly attributable, at least in part, to the calcium present in the product. In a preferred embodiment, the provider of a particular calcium-containing product communicates the obesity control benefits of calcium. The obesity-control information provided according to the subject invention maybe provided with, or without, information relating to other health benefits that may be attributed to calcium or other components of the product, which contains calcium. In a specific embodiment, information regarding calcium obesity-control benefits would be practiced by a commercial entity having a financial interest in the sale of a product, or a class of products, which contain calcium.

The document EP2168445A1 reports the method for obtaining a dietary fiber composition useful to produce functional food products as pasta, bakery products, breakfast cereals, snack or drink [51]. The method for treatment of bran to obtain a dietary fiber composition comprises the step of subjecting the bran to solubilization in the presence of an enzymatic mixture obtained from a culture of a *Trichoderma* strain, the culture being incubated on substrate containing at least 1% (w/v) of bran in conditions suitable for the production of lytic enzymes. It is also described a dietary fiber composition having prebiotic properties which contains a quantity of soluble fiber greater than about 2% by weight over the total dry weight of fibres and a polyphenols level accounting about to 0.15 mg of gallic acid per 100 g of fibers according to the Folin-Ciocalteus method. The present invention further refers to a dietary fiber composition having an increased soluble fiber content and its use in the preparation of high soluble fiber functional food with prebiotic properties. Such a problem has to be solved, according to the invention. According to an embodiment of the present invention, the bran present in the substrate is the same as the bran subjected to solubilization. Preferably, the *Trichoderma* strain is chosen between *T. reesei* strain 67 and *T. harzianum* strain T22. The bran preferably consists of durum wheat bran, chosen from the cereal processing by-products referred to as B&B50 and B&B70, their derivatives and mixtures thereof. Such brans are secondary products obtained from the decortication of durum wheat caryopses and represent particularly suitable and inexpensive substrates. The bran derived from the processing of the outer layers of the caryopses is removed by preliminary friction and/or abrasion operations before the milling of the same. A type of selected bran having a high fiber content, obtained from B&B70 was also tested, using additional selective steps with the aim of ascertaining whether the higher concentration was capable of more intensely stimulating growth of fungus, compared to the two types of selected bran. The term "enzymatic mixture" is herein used to indicate an extract of microbial culture containing at least one type of enzyme. Preferably, the enzymatic mixture contains enzymes chosen from the group comprising glucanases, xylanases, chitanases and cellulases, in particular endoglucanases and endoxylanases. The chosen *Trichoderma* strain is preferably grown on a substrate containing about 1% bran. The solubilization step comprises incubation of a reaction mixture comprising bran dispersed in distilled water, at a proper temperature. This particular composition of enzymes has proven to be surprisingly effective in the solubilization of insoluble bran fibers, thus allowing to obtain, by means of high concentrations of enzymatic extract of fungal origin, a dietary fiber composition composed of soluble fiber even up to 12.5%. It has been surprisingly found out that the dietary fiber composition obtained through the method of the present invention is not subjected to deterioration in terms of organoleptic properties compared to the starting bran. Furthermore, it has the advantage of having prebiotic properties and a high polyphenols level that could contribute to the protection against free radicals, thus playing a key role in atherosclerosis, cardiovascular and pulmonary diseases, arthritis and neurological disorders.

4.3. Prebiotic/Probiotic

The document WO2010081913A2 reports the production of a composition of plant based material e.g. glucose of cereal, obtained by hydrolyzing or transglucosylating glucose, hydrolyzing malto-oligosaccharides into glucose, oxidizing glucose and removing gluconic acid [52]. The present invention relates to a process for the production of a prebiotic composition, comprising the steps of (a) providing a plant based material wherein the plant is selected from the group consisting of cereals, legumes, tubers and mixtures thereof, wherein said plant based material comprises dietary fiber, optionally starchy material and optionally glucose, or wherein said plant based material comprises starchy mate-

rial, and optionally glucose; (b) hydrolyzing or tranglucosylating at least part of the dietary fiber into glucose and into at least one non-digestible oligosaccharide and optionally into at least one non-digestible polysaccharide, and optionally hydrolyzing and transglucosylating at least part of the starchy material or part of the malto-oligosaccharides into glucose and into at least one non-digestible oligosaccharide; (c) oxidizing at least part of the total glucose, consisting of said optional glucose of step (a) and said glucose obtained in step (b), to gluconic acid or a salt thereof; (d) removing at least part of said gluconic acid and/or a salt thereof obtained in step (c); thereby obtaining a composition comprising dietary fiber and gluconic acid or a salt thereof, wherein said dietary fiber comprises at least one non-digestible oligosaccharide and optionally at least one non-digestible polysaccharide.

The document WO2010051792A1 has for object of the invention the use of microtablets containing a dietary supplement e.g. probiotics and prebiotics, and/or a drug, as a food additive or feed additive for production of functional foods e.g. cookies and fruit bars, or functional feed [53]. In addition, the present invention also extends to methods for producing functional foods and feeds and also to foods and feeds, which comprise microtablets.

The document WO2010059022A1 reports the mixture for preparing a functional food with symbiotic action comprising strain of probiotic, prebiotic and nutrient solution for human consumption [54]. This mixture contains at least one probiotic strain selected from *Lactobacillus, Bifidus, Streptococcus* and/or yeasts; at least one prebiotic basically being inulin whereunto is added, a simple carbohydrate being glucose and/or a monosaccharide being sugar, and amines being vitamin E, pyridoxine chlorohydrate, folic acid, vitamin B12 and ascorbic acid, and proteins of animal or vegetable origin, with or without the presence of the nine essential amino acids, lipids, preferentially short-chain, omega, and short-chain carbohydrates.

The patent WO2011017040A1 describes a nutritional composition comprising fiber and probiotics [55]. Nutritional composition including fiber blends having a stable amount of probiotics and methods of making the nutritional composition are provided. In a general embodiment, the present disclosure provides a nutritional composition including a fiber blend having agglomerated of fiber particulates and probiotic with a water activity of less than about 0.15. The nutritional composition can be used as pharmaceutical formulation, nutritional formulation, dietary supplement, functional food and beverage product.

The document US7897185B1 has for object of invention the development of a cream cheese and methods of making it [56]. The technique for making the cream cheese product comprised: (i) providing a milkfat fluid comprising butterfat; (ii) pasteurizing the milkfat fluid; (iii) homogenizing the milkfat fluid; (iv) culturing bacteria in the milkfat fluid; (v) producing a cream cheese comprising live probiotic bacteria cultures.

The document US20100303778A1 mentions new *Saccharomyces cerevisiae* strain submitted to the National Collection of Cultures of Microorganisms, useful for preparation of food supplement, probiotic, functional food, cosmeceutical and/or active pharmaceutical ingredient [57]. The invention relates to novel yeast strains, to the yeasts resulting from these strains, to a composition containing at least one *Saccharomyces cerevisiae* yeast and/or derivatives of a yeast having a particular interest as a food additive and/or probiotic and/or functional food and/or nutraceutic and/or functional ingredient and/or cosmeceutical and/or pharmaceutical active agent. The invention also relates to the use in human and/or animal nutrition, or for the treatment or prevention of inflammatory diseases.

The document US20100203018A1 describes zinc-enriched biomass preparation used as probiotic agent for manufacturing probiotic composition (e.g. dietary products) by culturing microorganisms consisting of *Bifidobacterium animalis* subsp. *lactis* and/or *Streptococcus thermophilus* [58]. Furthermore, new microorganism strains are able to concentrate zinc within the cell in very high amounts are described.

The document WO2006039768A1 mentions a functional food containing probiotics and prebiotics, optionally as fermented soy-based food or beverage, or sweetened or aromatized, enriched with other functional agents in a balanced form [59]. The present invention relates to a fermented functional food based on soy containing probiotics and prebiotics and a process of production thereof. The functional food contains water-soluble soybean extract, water, probiotics, prebiotics, sweetening, optional food additives and functional agents. The process comprises the preparation of the inoculum and the fermentation medium, the inoculation with the probiotics, the fermentation, the optional treatments, and the packaging conditions.

The document US7101553B2 describes how to enhance immune responses by administration of a composition that includes a prebiotic, especially a combination of FOS and inulin [60]. The prebiotic carbohydrate formulation has to be used for enhancement of immune response and for prevention or treatment of measles. It is well known that prebiotics comprise carbohydrates and more specifically, oligosaccharides. Furthermore it is known that they have widely been used as functional food ingredients. They resist hydrolysis by enzymes of the human digestive tract, can reach the colon un-degraded and provide a carbohydrate substance particularly suited to the growth of bifidobacteria. Oligosaccharides may be produced from glucose, galactose, xylose, maltose, sucrose, lactose, starch, xylan, hemicellulose, inulin, or a mixture thereof. Purified commercially available products such as fructo-oligosaccharides contain greater than about 95% solids in the form of oligosaccharides. Measles is a major public health problem, infecting approximately 70 million children annually, and it is estimated that 2 million die each year from the disease itself or its complications. In addition to fever and rash, the consequences of measles include acute diarrhea or dysentery, pneumonia, encephalitis, and blindness due to acute vitamin A deficiency. It has been found that children fed a diet comprising a prebiotic formulation have a significantly enhanced immune response after vaccination than children fed a control diet without this prebiotic formulation. In a first aspect the present invention provides a composition comprising at least one prebiotic for

enhancement of an immune response. In a second aspect, the invention provides use of a prebiotic in the manufacture of a medicament or nutritional composition for enhancement of an immune response. In a third aspect, the invention provides use of a prebiotic or composition in the manufacture of a medicament or nutritional composition for the prevention or supportive treatment of measles. In a fourth aspect, the invention provides a method of enhancing an immune response which comprises administering an effective amount of a prebiotic or composition comprising at least one prebiotic. In a fifth aspect, the invention provides a method of prevention or supportive treatment of diseases such as measles which comprises administering an effective amount of a prebiotic or composition comprising at least one prebiotic. The composition may include a probiotic in addition to a prebiotic. Preferably, the probiotic is selected from the group consisting of *Bifidobacterium bifidum* and *Streptococcus thermophilus.* Preferably the *Bifidobacterium bifidum is Bifidobacterium lactis.* An advantage of the present invention is that it provides an elevated immune response after vaccination and therefore offers substantial protection until a second follow-up vaccine can be administered. Another advantage of the present invention is that it provides an elevated response to early measles vaccination, thus offering substantial protection against measles until a second measles vaccine is administrated. Moreover, it may be employed to enhance an immune response, e.g., protection against measles, by simple consumption of food before, during, and after the vaccination period. It will be appreciated that intravenous or subcutaneous administration of a drug requires expertise, and compared to oral administration it is not as safe, convenient or acceptable to the patient. In the light of these concerns, the invention provides the clear advantage of a nutritional and/or therapeutic product, which may be administered orally although other forms of administration can be used. In an embodiment, the nutritional composition comprises a milk-based cereal together with a prebiotic formulation. Preferably, the milk-based cereal is an infant cereal that acts as a carrier for the prebiotic formulation. The most preferred prebiotic comprises a mixture of fructo-oligosaccharides and inulin in the amounts by weight of 70% fructo-oligosaccharides and 30% inulin. If desired and advantageously, the composition may include a source of protein and/or a source of carbohydrate and/or a source of fat. Dietary protein is preferred as a source of protein. Dietary fibers can be included, if desired and when present, it comprises up to about 5% of the weight of the nutritional composition. The dietary fibers may be provided from any suitable origin, including for example soy, pea, oat, pectin, guar gum, gum arabic, FOS or a mixture thereof. Suitable vitamins and minerals may be included in the nutritional composition in an amount to meet the appropriate or desirable guidelines. The nutritional composition for enhancing immune response is preferably entirely administrable. If it is desired to produce a powdered nutritional formula, the homogenized mixture is transferred to a suitable drying apparatus such as a spray drier or freeze drier and converted to powder.

The document US20100028489A1 reports pre-fermented symbiotic matrix useful in pharmaceuticals, cosmetics, and foodstuffs e.g. drinks, contains cereals suspension, preferably oat, with encapsulated microorganisms, encapsulated prebiotics, and other food ingredients [61]. Prebiotics and their combination with probiotics in an encapsulated form are considered as an investment for food the industry, with the intention of maintaining their long-term stability and optimizing the nutritional qualities of the associated product. The present invention's object is also complementing the actual functional food market and solving problems inherent to the reduced shelf-life period of such foods due to loss of probiotic viability to values below the minimum limits needed, in order to promote biological activity. Furthermore, the present invention aims to improve the fermentative process conditions at different levels, reduce energy consumption, risk of contamination as well as promotion of long term microbial stability maintenance. The pre-fermented symbiotic matrix is designed, in particular, for those cases where intolerance and/or allergy to dairy products occur, but it is further applicable to the pharmaceutical, cosmetic and preferably food industries, including pet food. The products obtained possess sensorial characteristics that are identical to those produced by traditional fermentation processes. The process reveals a method for the improvement of fermentative process conditions at several levels such as (i) the continuous reutilization of the immobilized cells; (ii) the fermentation time reduction contributes to energy saving throughout the process, (iii) the reduction of contaminating risks and (iv) the long term maintenance of microbial stability.

The document US20100003370A1 discloses on functional sugar replacement. The invention is related to a functional food ingredient, which replaces sugar on a 1/1 weight and/or volume basis in food recipes containing sucrose, with a substantial caloric reduction in view [62]. The functional food replacement for sucrose, according to the present invention comprises prebiotic fibers and sweeteners, and possibly other non-selective fibers, minerals, vitamins and probiotic strains. More particularly, the present invention involves a solid or semi-solid functional sweetener that can be used to replace sugar in any preparation on a 1/1 weight basis and/or additionally on a 1/1 volume basis. Sugar is a popular sweetening additive in human food preparation. Popular feeding habits tend to show an over consumption of sugar. However, due to its elevated calorie content, high uptake of sugar is not recommended for dietary reasons. Further, people with diabetes need to control the intake of sugar. A high level of glucose in the blood is harmful. Even though the symptoms are not immediately severe, over time, uncontrolled high blood sugar levels can damage the smaller blood vessels, leading to complications including irreversible damage to the eyes and kidneys. Nerves and organs can also be damaged, as well as the ability to feel sensations and pain. Uncontrolled diabetes increases the risk of cardiovascular diseases such as heart attack and stroke. Therefore, sugar replacement solutions for popular foodstuffs are of high value. The present invention relates to a new concept with respect to healthy nutritional habits and functional foods. The basic and most important idea for this concept is that people no longer need to change their food habits in order to improve their health. Within the concept of the invention, it is possible to consume foods with positive health effects as simple and effective as possible, without giving up anything the consumer likes such as sweet or salty taste, palatable structure and texture of the food

products. The main goal is to replace those food ingredients that are used in the largest quantities, but at the same time present an insidious poison to even healthy human beings. One of the most important ingredients in this context is sugar. In this view, it is important to acknowledge the presence of complex microflora in the gastrointestinal tract, more specifically the colon. In the gastrointestinal tract, microorganisms are prevalent in the colon. It is known that microbes in the large intestine complete the digestion process on food components that are not digested in the small intestine, such as fibers and plant-derived food materials. The characteristics of these oligo- and polysaccharides are dependent on the saccharide composition, the bonds between the saccharides and the degree of polymerization.

Some of these fibers have prebiotic properties and are called prebiotic fibers or prebiotic oligo- or polysaccharides. They are mainly soluble oligo- and polysaccharides that are non digestible, which means that they are neither digested by human enzymes of the gastro-intestinal tract nor absorbed in the upper digestive tract. Thus, they arrive unchanged in the colon where they are at least partially fermented, mainly by beneficial bacteria present in the colon, such as *Bifidobacteria* and *Lactobacilli.* Hence, these beneficial bacteria utilize pre-biotic fibers as selective energy source for growth and proliferation in the colon. This effect is called prebiotic activity, referring to stimulation and/or activation of health promoting bacteria in the intestinal tract. Studies on humans have, for instance, confirmed that ingestion of moderate amounts of these pre-biotic fibers (from 5 g per day) results in a significant increase (up to 10 fold) of *Bifidobacteria* in the colon. During fermentation, these fibers are degraded and short chain fatty acids are produced, lowering pH levels and providing an energy source for growth and maintenance of large intestine cells. This process leads to differentiation of cancer cells, a vital step that is required before cancer cells can be killed. The pH lowering effect of the acid production results in an improved calcium and magnesium uptake, and simultaneously creates a harmful environment for pathogenic and putrefactive bacteria, such as *Clostridia*, *E. coli* or *Bacteroides.* Replacement of sugar by intense sweeteners is a serious problem in solid and semi-solid comestibles, because sucrose fulfils both a structural and sweetening function in these products. Preparation of low sugar or no sugar added products automatically faces the problem of replacing the bulk material in the product, which, in addition, should have at least the same functionality as the replaced sugar. Moreover, said replacement should provide essentially the same sweetness as sugar, at least the same functional effects as sugar on structure, texture, appearance and palatability of food preparation, but should also have some additional functionality such as health promoting effects and/or increased shelf life of processed products. Accordingly, the sugar substitute should not only replace sugar but should additionally offer a wide range of health effects while providing the human body with the required amounts of fibers, vitamins and minerals. To sum up, it can be stated that by using the sugar substitute, a better health should be obtained without making any concessions on neither taste nor structure. Preferably, at least one component of the bulking fiber composition is prebiotic. Moreover, it is preferred that at least one component is composed of mainly glucose units and at least one component is composed of mainly fructose units.

The document US20060252725A1 describes the use of prebiotics to prepare food, nutraceutical or pharmaceutical products for preventing or treating oxidative stress, especially caused by high fructose consumption [63]. The invention relates to the use of prebiotics for the preparation of food formulations, functional foods, or pharmaceutical compositions for preventing or treating oxidative stress in particular linked to the consumption of fructose. The invention also relates to a food preparation comprising simple carbohydrates, in particular fructose, in combination with prebiotics. A subject of the present invention is the use of prebiotics for preparation of functional compositions intended to prevent or treat oxidative stress, as the result of an imbalance in favor of pro-oxidant species relative to anti-oxidant species. Pro-oxidant species are generally free radicals and in particular oxygenated free radicals. Moreover, during inflammatory reaction, the stimulation of the phagocytes is also accompanied by the formation of free radicals. The anti-oxidant defenses of the organism use protein systems, such as superoxide dismutase, but also anti-oxidant compounds provided by food, such as vitamins C and E, or other nutrients, such as carotenoids, polyphenols or flavonoids. The inventors have in particular shown that foods that are too rich in sugars, and in particular in saccharose [64] and fructose [65], could cause significant oxidative stress. Because of their low production costs, syrups that are rich in fructose produced from corn are preferably used in sugary drinks. While fructose is naturally present in honey and in fruits where it is associated with many protective micronutrients, the consequences of an unrestricted increase of this carbohydrate in purified form on health are questionable. In fact, fructose has many properties that distinguish it from other sugars and the high intake of this carbohydrate could be responsible for undesirable metabolic effects. The present invention demonstrates that prebiotics and more particularly FOS can combat the oxidative stress resulting from an excess of fructose in food. The inventors have shown that the addition of prebiotics to the food intake, advantageously the addition of FOS, allowed a reduction in oxidative stress due in particular to a diet rich in sugars, and in particular fructose.

The document EP0998206A1 reports the nutritional supplement based on FOS and phyto-estrogens for treatment of menopause symptoms and prevention of osteoporosis [66]. The invention relates to a food preparation comprising a vegetal substance containing phyto-estrogen, consisting of crushed flaxseed containing lignan and soya flour containing isoflavonoids. In addition, the preparation contains a FOS with a prebiotic effect, such as inulin. The phyto-estrogen of the preparation is intended to act on menopause symptoms in women, and the purpose of FOS is to enhance intestinal activity by increasing the growth of *Bifidobacteria* in the intestines, thus promoting phyto-estrogen conversion and absorption in the intestines. In accordance with the invention, the preparation is a mixture of components in the form of a solid such as a mixture consisting of soya flour, crushed flaxseed, FOS and a flavoring and coloring vegetal substance, such as dried rosehip or lingonberry, that can be mixed to form a drink.

Soya has the characteristic of containing phyto-estrogens in abundance, more specifically daidzein and genistein, belonging to the isoflavonoids. Phyto-estrogens are hormone-like compounds occurring naturally in certain plants and greatly resembling the estrogens produced by the female organism. It is known that vegetal phyto-estrogens are converted in the intestines by the fermentation maintained by intestinal bacteria into substances having a similar effect as that of the estrogens produced by human organism. Thus, it is possible to alleviate problems connected with the menopause in women by adding phyto-estrogens to the food, substituting these for synthetic estrogen drugs. As a matter of fact, in countries where the food traditionally contains soybean products in abundance, women have significantly less menopause symptoms than in countries where the food is poor in phyto-estrogens. Phyto-estrogens prevent "hot flushes", prevent vaginal dryness and have also been confirmed to have an anti-depressive effect. Phyto-estrogens also delay bone fragility, thus reducing the risk of contracting osteoporosis. Except in soya, there is an abundance of phyto-estrogens in flaxseed, which contains secoisolariciresinol and matairesinol belonging to lignans. In addition, many other plants, such as some cereal crops, contain phyto-estrogens in minor concentrations. Owing to the known effects of phyto-estrogens mentioned above, there is an abundance of functional food preparations on the market, in which especially soya, but also flaxseed constitute a chief ingredient. A typical preparation consists of vegetal ingredients in the form of a finely or coarsely divided powder that contains soya flour or crushed flaxseed, or preferably both, and it is intended as a drink mixed in a liquid. Such a particulate preparation can also be used as a supplement to be mixed in other foodstuffs, such as yoghurt and various cereals and flakes. Besides phyto-estrogens, the preparation is rich in fibers and poly-unsaturated fatty acids, thus allowing the need for synthetic estrogen drugs to be avoided during the menopause. The invention aims at the joint effect of fructo-oligosaccharides and phyto-estrogens, so that the growth of useful Bifidobacteria in the intestines enhances the intestinal activity and thus boosts the conversion of phyto-estrogens into a form usable by the intestines and their absorption into the human body. Moreover, a special purpose of the invention is to provide a preparation that is easy to use as a food supplement, mixed in various foodstuffs or drinks. Thus, this preparation is characterized by being a mixture of components in the form of solid, finely divided particles, which contains FOS with a prebiotic effect combined with a vegetal substance containing phyto-estrogens, which consists of soya flour containing flavonoids and crushed flaxseed containing lignan. The preparation of the invention can be prepared by mixing vegetal components for instance in water, sour milk or juice, forming a drink consumed as such. With the adequate use of crushed flaxseed containing lignans and soya flour containing isoflavonoids, the phyto-estrogen concentration can be controlled within a proper range.

5. CURRENT & FUTURE DEVELOPMENTS

The high potential that functional foods can achieve on the world market is determined by the fact that the driving force of this sector is mainly the increasing basic awareness of the metabolic and physiological action of natural compounds, which bring better health benefits than conventional products when added to foods or when naturally present in them. The nutraceutical sector has become one of the most dynamic segments of the food industry. Many companies have invested in development, production and marketing of nutraceuticals. As the need to meet new challenges and new enquiries by consumers is increasing, the development of processes focused on optimisation of nutrition and improvement of eating habits also increases, the production of functional food is prevalently covered by multinational companies. On the other hand, SMEs are mostly active in the production on “niche-food products”, even if the business opportunities in that field is continuously increasing. The companies recognize the opportunity and urgency of aiming towards strengthening the tendency for innovation as a vehicle for the completion and/or consolidation of the process of internationalization, and towards the implementation of network and production chain integration strategies. The soaring of public health costs and the dissemination of preventive medicine are pulling consumers to deepen their knowledge of relations between food, health and well being. Consumers are increasingly aware about reducing health risks and improving their lifestyle through more correct eating habits. This awareness is also due to research results on food antioxidants, beneficial substances found in vegetables and crops, and probiotic microorganims. Functional foods are rich in many bioactive compounds, most of which shows antioxidant and/or anti-carcinogenic and anti-inflammatory properties. Particularly, anti-carcinogenic activity of those compounds is mediated by several molecular pathways, like antioxidant, anti-mutagenic activity, stimulation of immune defence system, modulation of antioxidant enzymes, modulation of hormonal system and genetic expression regulation in cell growth and apoptosis. Although these molecules are considered to possess chemo-preventive activity, their exact biochemical function is still unclear. Food nutritional qualities are determined by their natural constituents and components that are produced during processing and storing. Moreover, the increasing demand of fortified foods is raising new issues about the level of micronutrients and supplement ingestion, in terms of efficiency and food safety. An additional element of product innovation could be its optimization by micro and nano encapsulation. In fact, many functional ingredients are sensitive to environmental factors such as light, moisture, heat, etc. Moreover, if used for enrichment of cooked products may be damaged during the different stages of production. The functional ingredients may also react with other food components and/or add colors, aromas and undesirable flavors. For this reason, encapsulation can protect the component from mechanical and thermal damage. Further innovative elements concern development of alternative processes to traditional solvent extraction technology. The assessment of the nutraceutical quality of the elements and their preservation during storage is also a key aspect for a functional food. This will lead to identify the critical conditions for maintaining phytocomplexes properties in the final product (temperature, humidity, interaction of bioactive compounds with food matrices, etc.). Finally, cerebral mechanisms of subjective perception of functional foods and homeostasis of weight failure by many people are still unknown.

CONFLICT OF INTEREST

The author has no conflict of interest to declare.

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